



## Determination of Wool Follicle Characteristics of Iranian Sheep Breeds

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**ABSTRACT :** In this study wool follicle characteristics of Iranian sheep breeds were investigated. A total of 242 male and female sheep of different age from 5 breeds were studied. Samples of skin were taken from the right midside to determine follicle characteristics. Overall averages for males and females were  $3.7 \pm 0.1$  and  $3.5 \pm 0.1$  for S/P ratio;  $3.2 \pm 0.1$  and  $3.6 \pm 0.1$  for primary follicle density;  $12.1 \pm 0.7$  and  $13.8 \pm 0.4$  for secondary follicle density;  $15.3 \pm 0.7$  and  $17.4 \pm 0.4$  for total primary plus secondary follicle density;  $2.3 \pm 0.2$  and  $3.5 \pm 0.3$  for percentage of inactive secondary follicles. Significant differences were found in some follicle characteristics between sheep breeds. Results of this study showed that sheep breeds raised in different parts of Iran characterized by a low S/P ratio and follicle density could be classed as carpet wool breeds. (**Key Words :** Sheep, Wool, Follicle, Breeds, Iranian)

### INTRODUCTION

In 2008 there were about 53.8 million heads of sheep (FAO, 2008) in Iran with 27 well defined breeds. Sheep raising for wool production play an important role both as income to farmers and as important source of raw material for hand-woven carpet industry. Wool come second after meat production in the farmer's priority and sold in the local market. All sheep breeds belong to the fat-tailed, coarse carpet wool type except Zel breed which is a non fat-tail sheep. The fleece of sheep grows from specialized follicles in the skin (Nixon et al., 1991). Primary follicles bear hair which are characteristically medulated and coarse ( $>35 \mu\text{m}$ ) and provide a mechanical protection. Secondary follicles are more numerous than primary follicles and produce non-medulated fine fibre ( $<35 \mu\text{m}$ ) which provide thermal protection. To increase the production of wool both quantitatively and qualitatively it is obligatory to study

follicle characteristics responsible for the production of inner insulating non-medulated fine wool and outer guard medulated coarse hair. Definition of follicle characteristics are required to enable a better understanding of the variability of fibre types and the growth dynamics of fine and coarse fibres. Presently, little information is available either nationally or internationally on Iranian sheep follicle attributes. Accordingly, the present work was designed to identify follicle characteristics of Iranian sheep breeds which is of primary importance in exploiting the production potential and quality of fibre for the future utility.

### MATERIALS AND METHODS

#### Selection of animals

A total of 242 sheep (60 males and 182 females) of Afshari, Zandi, Mehrabani, Lori and Baluchi breeds respectively from Zanjan, Qom, Hamedan, Lorestan and South Khorasan provinces were used in this study. The sheep grazed all year but their diets were supplemented during winter with limited amount of forage and grain (containing  $15 \text{ g N kg}^{-1}$  dry matter and  $9.1 \text{ MJ}$ ) and were housed at night during severe weather conditions. Sheep were grouped into 4 age groups: 1, 2, 3 and 4 years old.

#### Skin sampling and staining

One sample of skin per sheep were taken from the right

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midside of animals at the end of spring. In order to facilitate skin sampling, sheep were restrained in a lateral position, the right midside of the animals was clipped and anaesthetized with 1% lignocaine. A 1-cm diameter trephine was used to make an incision to the connective tissue beneath the skin.

The skin section was raised with forceps and a hand-held scalpel blade was used to cut parallel to the skin surface through the connective tissue, removing the skin section. The samples were then placed in small mell individual baskets and dehydrated through a series of graded ethanols, cleared in histoclear using a Citadel tissue processor (Histokinette 200, Cambridge Instruments Company). Processed skin samples were embedded in paraffin using Leukhardt blocks. Embedded skin samples were sectioned in transverse plane to the follicle line at 8  $\mu$ m using a base sledge microtome (Model Leica rm 213s, Nussloch, Germany). Approximately 60 sections were cut per sample, but only every fifth section was retained. Twelve sections were retained per sample and transferred to slides. Before staining all sections were deparaffinised and immersed in histoclear for 2 minutes and rehydrated in a graded series of ethanols to water. A special tetrachrome stain 'sacpic' (Auber, 1952) was used to demonstrate follicular tissue compounds.

#### Follicle density, S/P ratio and follicle activity (morphology)

For each sheep at least 25-30 follicle groups per skin sample were used to estimate follicle S/P ratio. Follicle density per 1 square millimeter of skin was counted (Clark, 1960). Primary and secondary follicles were identified through the associated gland structures. To determine the percentage of inactive secondary follicles of skin samples taken, approximately 300 follicles were counted per midside skin sample from randomly selected follicle groups as described by Nixon (1993). The basic criteria for distinguishing an active from an inactive follicle is the presence of fibre and inner root sheath cells. In an active follicle stained with SACPIC staining method a yellow

stain fibre is surrounded by a red stained inner root sheath, while in an inactive follicle these structures are either absent or disrupted. In a telogen follicle the outer root sheath cells are often columnar and radially or spirally arranged in contrast to the rounded shape of this cell type and their arrangement during anagen.

#### Statistical analysis

Analysis of variance was performed using a general linear model (GLM) of SAS package (SAS, 1996). Differences between means were tested using Duncan's new multiple range test. The statistical model used for cashmere goats of different age, sex and breed was:

$$Y_{ijk} = \mu + \alpha_i + S_j + B_k + (\alpha S)_{ij} + (\alpha B)_{ik} + (SB)_{jk} + (\alpha SB)_{ijk} + \varepsilon_{ijk}$$

where:  $Y_{ijk}$ : dependent variables;  $\mu$ : the overall mean;  $\alpha_i$ : the effect of age ( $i = 1, 2, 3, 4$ );  $S_j$ : the effect of sex ( $j = 1, 2$ );  $B_k$ : the effect of breed ( $k = 1, 2, 3, 4, 5$ ),  $\varepsilon_{ijk}$ : residual error;  $(\alpha S)_{ij}$ : interaction between age and sex groups;  $(\alpha B)_{ik}$ : interaction between age and breed groups;  $(SB)_{jk}$ : interaction between sex and breed groups;  $(\alpha SB)_{ijk}$ : interaction between age, sex and breed groups.

All values were expressed as least square means  $\pm$  SEM with  $p < 0.05$  was considered to be statistically significant.

## RESULTS

#### Secondary to primary ratio (S/P)

There was no significant difference in the mean S/P ratio between male and female sheep (Table 1). Mean S/P ratio of male and female sheep was  $3.7 \pm 0.1$  and  $3.5 \pm 0.1$  respectively. Significant difference in the mean S/P ratio was found between different sheep breeds (Table 1). Average S/P ratio of Afshari, Zandi, Mehrabani, Lori and Baluchi breeds were  $3.2 \pm 0.1$ ,  $3.9 \pm 0.1$ ,  $2.9 \pm 0.1$ ,  $3.8 \pm 0.2$  and  $4.0 \pm 0.1$  respectively. There was significant interaction

**Table 1.** Least square means and standard errors of sex and age groups of sheep breeds of Iran for secondary to primary (S/P) follicle ratio, primary (P) follicle density, secondary (S) follicle density, primary plus secondary (P+S) follicle density and percentage of secondary (S) inactive follicles

Follicle characteristics	Sex		Age				Breed							
	Male	Female	1	2	3	4	Afshari	Zandi	Mehrabani	Lori	Balouchi			
	60	182	74	45	69	54	60	54	41	36	51			
S/P	NS	$3.7 \pm 0.1$	$3.5 \pm 0.1$	NS	$3.6 \pm 0.1$	$3.4 \pm 0.2$	$3.6 \pm 0.1$	$3.5 \pm 0.2$	*	$3.2 \pm 0.1^c$	$3.9 \pm 0.1^{ab}$	$2.9 \pm 0.1^d$	$3.8 \pm 0.2^b$	$4.0 \pm 0.1^a$
P density	*	$3.2 \pm 0.1^a$	$3.6 \pm 0.1^b$	NS	$3.5 \pm 0.1$	$3.6 \pm 0.2$	$3.3 \pm 0.1$	$3.4 \pm 0.1$	*	$3.3 \pm 0.1^b$	$3.7 \pm 0.2^a$	$2.7 \pm 0.1^c$	$3.7 \pm 0.2^a$	$3.9 \pm 0.1^a$
S density	**	$12.1 \pm 0.7^a$	$13.8 \pm 0.4^b$	**	$14 \pm 0.6^a$	$14.7 \pm 0.9^a$	$12.5 \pm 0.6^b$	$12.5 \pm 0.6^b$	*	$10.4 \pm 0.3^c$	$15.1 \pm 0.8^b$	$8.0 \pm 0.3^c$	$15.8 \pm 0.8^b$	$17.6 \pm 0.5^a$
P+S density	**	$15.3 \pm 0.7^a$	$17.4 \pm 0.4^b$	NS	$17.5 \pm 0.7$	$18.2 \pm 1.1$	$16.0 \pm 0.7$	$16.0 \pm 0.7$	*	$13.7 \pm 0.3^c$	$18.7 \pm 0.9^b$	$10.7 \pm 0.3^d$	$19.8 \pm 0.9^{ab}$	$21.5 \pm 0.6^a$
Inactive S (%)	**	$2.3 \pm 0.2^b$	$3.5 \pm 0.3^a$	**	$2.8 \pm 0.4^{ab}$	$2.5 \pm 0.4^b$	$3.4 \pm 0.3^a$	$4 \pm 0.6^c$	*	$7.2 \pm 0.6^a$	$1.4 \pm 0.1^c$	$2.5 \pm 0.1^b$	$2.1 \pm 0.2^{bc}$	$1.6 \pm 0.1^{bc}$

<sup>a,b,c,d</sup> Within rows, mean without a common superscript differ at ( $p < 0.05$ ).

between sex and breed in S/P ratio.

It was observed that follicle groups consisted of 3 primary follicles associated with secondary follicles (Figures 1 and 2). The basic criterion for distinguishing a primary follicle from a secondary follicle is the presence of sweat gland in primary follicle. Secondary follicles do not bear sweat glands.

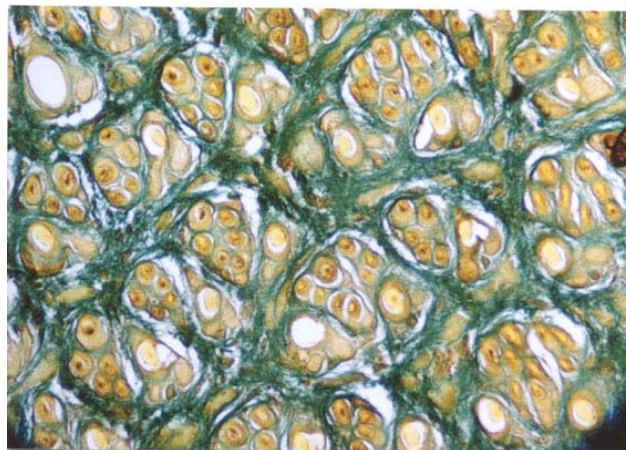
#### Follicle density

Mean primary, secondary and total primary plus secondary follicle density of male sheep was  $3.2 \pm 0.1$ ,  $12.1 \pm 0.7$  and  $15.3 \pm 0.7$  and for female sheep was  $3.6 \pm 0.1$ ,  $13.8 \pm 0.4$  and  $17.4 \pm 0.4$ . Males had significantly higher primary, secondary and total plus secondary follicle densities than females (Table 1). There was significant interaction between breed and age in secondary follicle density.

#### Secondary follicle inactivity

There was significant difference in the mean percentage of inactive secondary follicles of male and female sheep. Mean percentage of inactive secondary follicles of male and female sheep was  $2.3 \pm 0.2$  and  $3.5 \pm 0.3$  respectively. Afshari sheep breed had significantly higher percentage of secondary follicle inactivity. Percentage of inactive secondary follicles of Afshari, Zandi, Mehrabani, Lori, and Balouchi breeds was  $7.2 \pm 0.6$ ,  $1.4 \pm 0.1$ ,  $2.5 \pm 0.1$ ,  $2.1 \pm 0.2$  and  $1.6 \pm 0.1$ . Wide variation of follicle inactivity existed between individual sheep of Afshari breed ranging from a minimum of 1% to a maximum value of 30%.

Fibre shedding in Afshari breed was noticeable and started from neck extending to belly and rump areas as a result of follicle shutdown. Structure of fiber, inner root sheath and outer root sheath cells in inactive follicles were either absent or disrupted. In such follicles the outer root



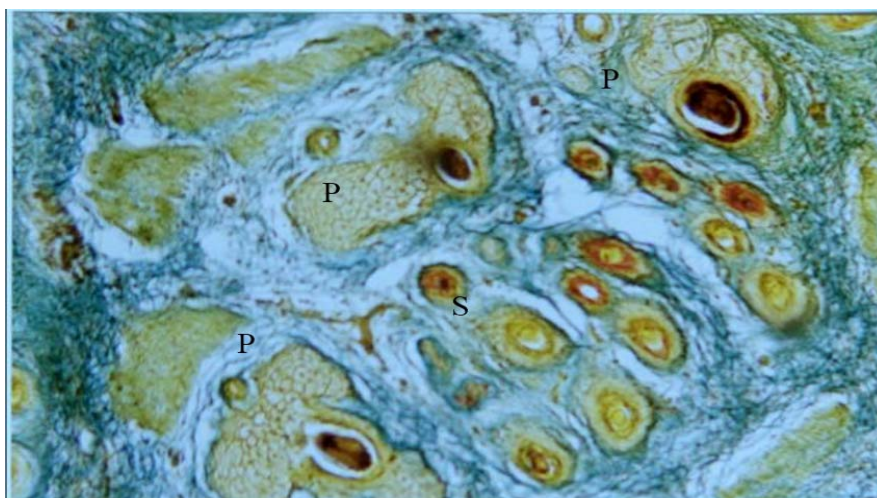
**Figure 1.** Transverse section through the skin sample (at the sebaceous gland level) indicating follicle groups.

sheath cells were often columnar and radially or spirally arranged in contrast to the randomly arranged cells in normal follicles.

#### DISCUSSION

Iranian sheep skin follicle group consisting of 3 discernable primary follicles and several secondaries is similar to that of Australian (Champion and Robards, 2000) and New Zealand (Chapman and Ward, 1979). The results of this study indicated that overall mean S/P ratio of Iranian sheep was  $3.2 \pm 0.1$  which is lower than Australian carpet wool sheep Carpetmaster ( $6.8 \pm 0.4$ ), Drysdale ( $5.8 \pm 0.6$ ), Elliotdale ( $6.6 \pm 0.8$ ), and Tukidale ( $6.7 \pm 0.6$ ) (Champion and Robards, 2000).

The similar S/P ratios in the follicle populations for the five sheep breeds largely explains the visual similarity of staple structure and density in their fleeces confirming



**Figure 2.** Transverse section through the skin sample (at the sebaceous gland level) indicating a follicle group consisting of 3 primary (P) follicles associated with secondary (S) follicles.

follicle studies in carpet wool sheep in Australia (Champion and Robards, 2000) and New Zealand (Chapman and Ward, 1979).

Follicle attributes vary between sheep breeds; S/P ratio being highest in Baluchi ( $4.0 \pm 0.1$ ) and lowest in Mehrabani ( $2.9 \pm 0.1$ ) which is within the range of values reported for carpet wool sheep breeds (Chapman and Ward, 1979). However these values are slightly lower than Australian Carpetmaster, Drysdale, Elliotdale, Tukidale (Champion and Robards, 2000) Suffolk and Ryeland (Carter and Clark, 1957) and New Zealand specialty carpet wool sheep breeds (Chapman and Ward, 1979) but are similar to more primitive Scottish Blackface, Welsh mountain, Swaledale and Wiltshire British sheep breeds (Chapman and Ward, 1979). Higher S/P ratio values reported for Australian and New Zealand carpet wool sheep breeds may reflect genetic gain due to selective breeding for wool growth characteristics over the last 50 years as increase in S/P ratio due to selection for fleece-weight, fibre diameter and live weight have been reported in sheep (Hynd et al., 1989). British breeds have coarse fleeces similar to that of Iranian sheep breeds in terms of fibre diameter, overall level of medullation and fleece structure.

The sheep's hair follicle group consisting of 3 primary follicles and several secondaries is similar to that of cashmere and mohair goat breeds (Ansari-Renani, 2010) and South American camelids (Frank et al., 1998), Bolivian Llamas (Lusky et al., 2004) and Peruvian Vicuna (Hoffman, 2004) but different from *Camelus dromedarius* (Ansari-Renani, 2008; 2010) where more than 3 primary follicles were found per follicle group.

Follicle density values recorded in this study highlight the similarities in skin and follicle populations of sheep breeds in Iran. The higher total follicle density in the Baluchi's (21.5) compared to Mehrabani (10.7) and Afshari (13.7) was due to higher secondaries. Afshari and Mehrabani breeds have much coarser fleeces in terms of fibre diameter and contains less fibre density than Baluchi and Lori breeds which have higher secondary and total follicle density. The coarseness and fleece structure of Iranian carpet wool breeds is not due to primary follicle density but rather secondary follicle density which in turn causes lower fibre diameter, decreased level of medullation or variability in the fibre length and diameter within the staple.

The average number of hair follicles per square millimeter of skin of male and female sheep was 15.3 and 17.4 respectively which is lower than *dromedarius* (30.1), *bactrianus* (32.8) camels (Ansari-Renani, 2010) and cashmere goats (36.19) (Ansari-Renani, 2011) but higher than that recorded in buffaloes (3.4) (El-Shafie, 1954). Criteria for estimating camel S/P ratio was different from that in cattle (Carter and Dowling, 1954) buffaloes (El-

Shafie, 1954) and rabbits (Ahmed, 1996) since all hair follicles in such species are primaries, i.e. each follicle is associated with a holocrine sebaceous gland, and apocrine sweat gland and an arrector pilli muscle with no discernible follicle group but in sheep of present study both primary and secondary follicles were present in discernible follicle groups.

Mean percentage of inactive secondary follicle was highest in Afshari (7.2) and lowest in Zandi (1.4). Secondary follicle inactivity is also common in double-coated British sheep breeds such as primitive Wiltshire and Soay sheep (Slee, 1959; 1963) and feral sheep such as Merino breed in Arapawa Island (Orwin and Whitaker, 1984) however the level of follicle inactivity which causes complete wool casting in latter breeds is much higher. In contrast, single-coated sheep breeds such as modern Merinos (Ryder, 1967) the incidence of hair follicle inactivity is very low. Large variations existed between individual sheep of this study in the number of inactive follicles ranging indicating large genetic variation in susceptibility to follicle inactivity.

Due to high percentage of follicle inactivity in Afshari sheep breed it was observed that shedding commenced on the neck, chest and shoulders and spreaded to the back and rump. This sequential, bilaterally-symmetric pattern has been also reported in cashmere goats (Ansari-Renani, 2011) and double coated Wiltshire sheep (Slee, 1959; 1963). Unlike natural shedding, cortisol injected Merino sheep, shedding of fibre starts from rump and belly areas extending to shoulders (Ansari-Renani and Hynd, 2001; Ansari-Renani et al., 2007). Follicle culture studies also indicated similarities of inactivity (Ansari-Renani and Hynd, 2004) *in vitro* in response to different concentrations of EGF and supra physiological doses of cortisol.

It has been indicated that onset of follicle inactivity is influenced by photoperiod being initiated in short day season and start activity in long day season (Hart et al., 1963; Ling, 1970; Panaretto, 1979). Changes in photoperiod modify neuro-secretory rhythms via the pineal gland, which ultimately affect the initiation of hair growth (Rougeot et al., 1984; Lynch and Russel, 1990) and follicle activity.

## CONCLUSION

It is apparent that there are differences between primary and secondary follicle populations in the way the fibres they produce contribute to different fleece structures such as fibre diameter, staple length and the level of medullation. However it can be concluded that S/P ratio and follicle density of Iranian sheep breeds studied fall within the range of common carpet wool sheep breeds and may be recommended for carpet wool production.

It was indicated that Afshari breed had highest percentage of inactive secondary follicles. Wool production of this sheep may be maximized by adopting suitable time of shearing and selective breeding schemes.

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